SUMMARY STATEMENT

PROGRAM CONTACT: [redacted] **(Privileged Communication)** ***Release Date:*** **12/19/2019**
Revised Date:

Application Number: **1 R01 DC018745-01**

Principal Investigator

LOMVARDAS, STAVROS

Applicant Organization: COLUMBIA UNIVERSITY HEALTH SCIENCES

Review Group: **ZRG1 IFCN-U (02)**
Center for Scientific Review Special Emphasis Panel
Member Conflict: Sleep, Movement, Mood and Olfaction

Meeting Date: **11/19/2019** ***RFA/PA:*** **PA19-056**
Council: **JAN 2020** ***PCC:*** **CS04**
Requested Start: **04/01/2020**

Project Title: **Principles of zonal olfactory receptor gene expression**

SRG Action: **Impact Score:13 Percentile:1 #**
Next Steps: **Visit https://grants.nih.gov/grants/next_steps.htm**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **30-Vertebrate animals involved - no SRG concerns noted**

Project Year	Direct Costs Requested	Estimated Total Cost
1	[redacted]	[redacted]
2		
3		
4		
5		
TOTAL	[redacted]	[redacted]

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

1R01DC018745-01 Lomvardas, Stavros

RESUME AND SUMMARY OF DISCUSSION: This is an R01 application from an established investigator seeking to elucidate the mechanisms underlying the zonal expression patterns of specific olfactory receptor (OR) genes in the olfactory epithelium. The investigator will use an array of state-of-the-art tools, including Cut and Run, to establish the genomic targets of different transcription factors, HiC, deep sequencing, and 11 different mouse lines, as well as CRISPR/dCas9 approaches, to parse the epigenetic role of NFI transcription factors and examine the influence of the zonal repressive mark H3K79me3 to explicate their collective critical contributions to zonal OR expression. During the discussion, there was considerable excitement for this application. The panelists remarked on the many strengths of the application, including the powerful, innovative techniques that will be applied to the research goals, the high value of the research goals for the field, the convincing, high quality preliminary data, and the “gorgeous” experiments proposed. Further, the panelists commented on the superb investigative team. Only two weaknesses were raised by the panelists: (i) whether the proposed full conditional knockout of three independently segregating alleles, using an independent inducible driver, would yield artifacts affecting interpretation, and (ii) a concern over whether the number of replicates were sufficient for the proposed analyses and interpretations, particularly with respect to sex as a biological variable. Overall, however, these weaknesses did not significantly affect the study section’s enthusiasm for this exceptional, high impact application that was considered likely to advance fundamentally our understanding of the mechanisms by which activating and repressive interchromosomal interactions incorporate feedback signals to regulate selective zonal transcription of a particular OR in the olfactory epithelium.

DESCRIPTION (provided by applicant): The monogenic, monoallelic, and seemingly stochastic transcriptional choice of one out of > 1000 olfactory receptor (OR) genes remained elusive for decades after the discovery of the largest mammalian gene family. However, in the past few years we obtained significant understanding on the molecular underpinnings of this enigmatic gene regulatory process. Specifically, we showed that OR gene clusters become heterochromatic at the early stages of olfactory sensory neuron (OSN) differentiation and then they aggregate in distinct nuclear compartments that assure their stable repression. As a result of this interchromosomal convergence, intergenic OR enhancers (known as Greek Islands) that are found in most OR gene clusters come in close nuclear proximity and form a multi-chromosomal super-enhancer that in each OSN associates with the transcriptionally active OR allele. The formation of the Greek Island hub is dependent upon the recruitment of the adaptor protein Ldb1, which is essential for the stable interchromosomal interactions between Greek Islands and for OR transcription. This intricate network of activating and repressive interchromosomal interactions, together with a feedback signal elicited by the expression of the chosen OR, likely generate the regulatory framework for transcriptional singularity. However, what remains unknown how this seemingly stochastic process operates under deterministic restrictions related to the spatial location of the OSN along the dorsoventral and apicobasal axes of the MOE. These restrictions, known as zonal pattern of OR expression, restrict the expression of each OR gene in one of five zones of expression. Here we identified putative mechanisms of zonal restriction, by uncovering the molecular mechanisms that enable only zone 5 ORs to be expressed in zone 5. We show that transcription factors of the NFI family enable the transcriptional activation of zone 5 ORs, by mediating the recruitment of these ORs to the interchromosomal OR compartment. Moreover, we show that the repressive histone modification H3K79me3 prevents the expression of out of zone ORs, possibly under the control of NFI factors, as well. We propose experiments that will decipher which NFI factors are required and sufficient for specification of zone 5 transcription programs, and experiment that will determine how NFI proteins accomplish these zonal restrictions. Our experiments will reveal novel mechanisms of regulation of nuclear architecture and will uncover generally applicable principles for the regulation of developmental patterning.

PUBLIC HEALTH RELEVANCE: Developmental patterning is a crucial biological mechanism perturbation of which are related to a plethora of developmental disorders. Developmental patterning in the olfactory epithelium, as manifested by the zonal expression of olfactory receptor genes remains mysterious for decades. Here, we describe genetic and epigenetic factors regulating zonal olfactory receptor expression, and we seek to uncover regulatory principles that are applicable to other systems undergoing developmental patterning.

CRITIQUE 1

Significance: 1
Investigator(s): 1
Innovation: 1
Approach: 1
Environment: 1

Overall Impact: The experimental program addressed by this application is focused on how olfactory sensory neurons express different odorant receptors in different regions of the olfactory epithelium. Recent studies, largely from the Principal Investigator's laboratory, have elucidated a complex multi enhancer complex that activates transcription from singular OR loci, followed by a feedback mechanism that helps to keep all other OR loci silent. In this application very promising preliminary studies suggest that the transcription factors NFIA, B, and X contribute to both the selective expression of olfactory epithelium Zone 5 ORs and the repression of Zone 2 and 3 ORs. The Aims of this application are designed to better understand NFIs' roles in zonal OR expression. A triple deletion of all three NFIs perturbs zonal OR expression. In Aim 1, each NFI will be tested individually for its ability to perturb expression through loss of function experiments, and its sufficiency for perturbation through gain of function experiments. This is worthwhile as different NFI factors may have specialized roles. Aim 2 addresses the mechanisms through which NFIs regulate zonal OR expression. In one series of experiments their direct binding sites will be probed using a method named 'cut and run'. In a second series of experiments interchromosomal contacts will be assayed using HiC in normal and NSI mutant OSNs collected from specific zones of the epithelium. One potential outcome is that zone appropriate OR to OR enhancer contacts will be detected, and that these will be disrupted in some of the knockout animals produced in Aim1. In a third series of experiments a candidate suppressive epigenetic mark, H3K79me3, will be tested for its contribution to zonal OR expression with an OSN knockout of the enzyme that produces it, and by determining whether any of the NFIs contribute to its deposition in zones that would otherwise be available for OR expression.

This is an outstanding application in every way. The biological problem it addresses is fundamental to understanding olfactory system function, it builds upon the seminal contributions the Principal Investigator has already made towards understanding OR gene expression, its preliminary findings are extensive, informative and provide a strong basis for the proposed experiments, and the experimental approach is innovative, powerful, and will almost certainly yield exciting results.

1. Significance
Strengths

- Understanding the mechanisms of the zonal expression patterns of specific OR genes in the olfactory epithelium is fundamental to understanding the development and function of the system. This is largely unexplored territory and an advance would have the potential to be of more general developmental interest.

Weaknesses

- None noted.

2. Investigator(s)

Strengths

- This Principal Investigator has been one of the primary leaders in efforts to understand how OR gene expression is controlled, especially through his laboratory's discovery of a complex multi-enhancer-based mechanism of gene choice and activation.
- This laboratory, by building on its previous studies and using some of the leading-edge technologies it has mastered, is in a unique position to address the mechanism of zonal OR expression.

Weaknesses

- None noted.

3. Innovation

Strengths

- There is a growing appreciation that epigenetic mechanisms control developmental pathways and cell identities. This research plan explores epigenetic contributions to an important regional pattern of gene expression whose origins are as yet unknown
- The investigator's laboratory has pioneered the use of HiC, deep sequencing, and other advanced techniques to solve a fundamental and fascinating developmental and genetic problem.

Weaknesses

- None noted.

4. Approach

Strengths

- The preliminary findings reported in the proposal are unusually strong and informative. They demonstrate the technical ability of the laboratory to perform the proposed experiments and point the research in what looks like a very promising direction. Not only are the NSI transcription factors identified as candidate contributors to zonal OR expression, but preliminary tests of necessity demonstrate their involvement. Target NSI motifs are identified in and enriched in zone 5 OR promoter sequences and NSIA binds general OR-activating enhancer sequences. The H3K79me3 suppressive mark is shown to be deposited in a zone dependent manner.
- It is noteworthy that an attractive and ultimately testable model for regional OR choice is presented in which ORs appropriate for a particular zone are made available for expression and then silenced, starting with zone 1 and then sequentially through zone 5.
- Each of the proposed experiments appears technically feasible for this laboratory and addresses a specific clearly formulated question. Reasonable alternative approaches are mentioned where appropriate.

Weaknesses

- From a purely technical perspective some of the proposed experiments may be challenging, but this lab has shown through past publications and the preliminary findings that they should be capable of accomplishing most of what they propose.

5. Environment

Strengths

- The Principal Investigator is situated in an institution with first rate resources and renowned Neuroscience and Genetics faculty.
- The high quality and significance of the publications coming from the Principal Investigator's laboratory over the past several years attest to the suitability of the research environment.

Weaknesses

- None noted.

Protections for Human Subjects
Not Applicable (No Human Subjects)

Vertebrate Animals
Acceptable

Biohazards
Acceptable (No Biohazards)

Budget and Period of Support
Recommend as Requested

CRITIQUE 2

Significance: 2
Investigator(s): 1
Innovation: 2
Approach: 2
Environment: 1

Overall Impact: The intellectual formulation of this application places the details of transcriptional regulation of zonally restricted odorant receptor expression into a very clear and compelling biological framework. The identification of a family of transcription factors, the NFIs, that may be key determinants of expression of multiple genes within a single zone provides a starting point to discern several mechanistic dimensions of patterned gene expression. Some concern is raised by the multi-allelic/ conditional recombination approaches necessary to test some of the hypotheses and the potential for low yield from these experiments and also genetic artifacts that reflect the technique but nevertheless influence execution and interpretation of the data. One wishes that the Principal Investigator had dealt more with this technical issue.

1. Significance
Strengths

- This application considers the mechanism and biological significance of zone restricted expression of odorant receptors based upon its transcriptional and chromosomal regulation and its expression as an example of patterned gene expression.
- The application places the problem of odorant receptor gene expression, and by extension coordinated expression of multiple genes in a distinct domain, in the appropriate context—how chromatin confirmation/architecture may or may not regulate this critical feature of morphogenesis and tissue architecture.

Weaknesses

- The application focuses on the mechanism of transcriptional control for patterning, but does not discuss whether the experiments and potential results address issues of the functional/ behavioral consequences of this developmental/cellular regulation.

2. Investigator(s)
Strengths

- The Principal Investigator has outstanding accomplishments in the field of transcriptional regulation and in the study of the olfactory epithelium as a model for highly regulated gene expression.

Weaknesses

- None noted.

3. Innovation

Strengths

- The focus on chromatin architecture, its regulation by identified transcription factors and the integrated contribution to patterning of these mechanisms is truly innovative.

Weaknesses

- The ambitious use of multi-allelic conditional inactivation to fully eliminate a set of related factors is technically demanding, but one wonders about the potential for artifact in this approach that takes a “cutting edge” technique to near to its known limits (full conditional knock out of 3 independently segregating alleles using an independent inducible driver).

4. Approach

Strengths

- The experiments to evaluate positive and negative regulation (expression versus exclusion of zonal OR genes) are particularly well designed and will yield high resolution data both in terms of developmental output, and the underlying mechanism of interchromosomal interaction and chromatin architecture in determining patterns of gene expression.

Weaknesses

- The multi-allelic, cell class specific recombination experiments though theoretically compelling have the potential for artifact and also resource-intensive but still low or slow yield data because of low probability of obtaining the needed genotypes. It would have been good to address these issues more thoroughly.

5. Environment

Strengths

- The environment at the Zuckerman Institute of Columbia University is outstanding in all ways for the completion of this work.

Weaknesses

- None noted.

Protections for Human Subjects

Not Applicable (No Human Subjects)

Vertebrate Animals

Acceptable

Biohazards

Acceptable (No Biohazards)

Budget and Period of Support

Recommend as Requested

CRITIQUE 3

Significance: 2
Investigator(s): 1
Innovation: 2
Approach: 2
Environment: 1

Overall Impact: This application's main goal is to elucidate the mechanisms underlying the specific zonal expression of odorant receptors in the mouse olfactory system. This mechanism has been very hard to determine, and thus the successful completion of this project will significantly advance the field. It is innovative in that it proposes a novel mechanism and uses cutting edge technology as well. The Principal Investigator is a leader in the field and has made important contributions to the mechanisms underlying singular expression of odorant receptors. His research environment is excellent, with numerous core facilities and there are exceptional opportunities to interact with prominent scientists. He presents convincing preliminary data identifying a zone 5 specific NFI motif on zone 5 OR promotors to support the research. A negative is that there are too few replicates to examine for sex differences if performed as proposed (4 replicates, 2 male and 2 female, which the Principal Investigator proposes to examine for sex differences prior to combining the data).

Protections for Human Subjects
Not Applicable (No Human Subjects)

Vertebrate Animals
YES, all criteria addressed

Biohazards
Acceptable

Resource Sharing Plans
Acceptable

Authentication of Key Biological and/or Chemical Resources
Acceptable

Budget and Period of Support
Recommend as Requested

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

VERTEBRATE ANIMALS: ACCEPTABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Footnotes for 1 R01 DC018745-01; PI Name: Lomvardas, Stavros

Ad hoc or special section application percentiled against "Total CSR" base.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-

14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

The roster for this review meeting is displayed as an aggregated roster that includes reviewers from multiple CSR Special Emphasis Panels of the SEP Aggregated Roster IFCN IRG